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To Study the Significance of Micronuclei and it's Scoring in Effusion Fluids

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Abstract: ***Background : Micronuclei are extra-nuclear bodies which contains fragments of damaged chromosomes and/or whole chromosome that were not incorporated into the nucleus during anaphase stage of cell division, as they lack centromere. MN formation is an important mechanism for chromosome loss in the nucleus. [08] Chromosomal instability leads to formation of MN which plays an important role in carcinogenesis. [09] MN scoring in effusions can be done to differentiate benign and malignant effusions. Aim of the study was to score MN in effusion fluids and to differentiate malignant effusions from benign effusions on the basis of MN score. Materials & Methods: A total 113 samples of ascitic and pleural effusions received for fluid cytology were studied. These fluid smears were stained with conventional papanicolaou stain. Number of micronuclei present per 1000 well preserved cells were counted under light microscopy. Result: Mean micronuclei score in malignant and benign ascitic effusions were 5.58 and 2.72 respectively, while in pleural effusions 4.0 and 1.8 respectively. Mann Whitney test showed that this difference was statistically significant (P ≤ 0.001). Conclusion: Micronuclei scoring can be used as a biomarker of chromosomal instability in routine reporting in distinguishing between malignant and benign effusions. MN scoring may be a simple, easy, alternative diagnostic tool to distinguish between benign & malignant effusions in low resource setting.***

Keywords: Fluid cytology, malignancy, micronuclei, score

1. Introduction

Micronuclei are extra-nuclear bodies which contains fragments of damaged chromosomes and/or whole chromosome that were not incorporated into nucleus during anaphase stage of cell division, as they lack centromere. Micronucleus is round to oval in shape having mean diameter of less than one third of primary nucleus.[01] MN formation is an important mechanism for chromosome loss in the nucleus. [08] Chromosomal instability leads to formation of MN which plays an important role in carcinogenesis. [09] A close relationship was found between micronuclei number and presence of chromosomal abnormalities and mutagen activity.[02] Cytological analysis is a less expensive way for assessing the cause of effusion. [03] Presence of micronucleus within cell denotes genomic instability, aneuploidy and has been associated with cancers, e.g., by studying cells in effusion fluids, exfoliated buccal cells, cervical smears and peripheral lymphocytes to detect damage chromosomal damage.[01,03,04]

A buildup of fluid in the bodily cavities is referred to as an effusion. [10] The parameters looked for in an effusion fluid sample are type of cells, abnormal increase in cells, arrangement, sizes, and its variability including cytoplasmic features as well as nuclear features. [5, 11] Ascites is the collection of fluid in the peritoneal cavity. A malignant ascitic effusion commonly associated with colorectal or ovarian carcinoma.[07] Pleural effusion is the collection of the fluid in the pleural cavity. Pleural effusion is most commonly associated with adenocarcinoma of the lung. [12,13]

The diagnostic yield of pleural fluid cytology ranges from 40 to 87%, while that of ascitic fluid cytology ranges from 56.7% to 60%. [06, 12, 13, 14]. Differentiating reactive cells from malignant cells is of great importance in management of cancer and its prognosis. Micronucleus can be identified alternatively by DNA cytometry, Fluorescence in situ hybridization (FISH) to assess genomic instability [21, 22]

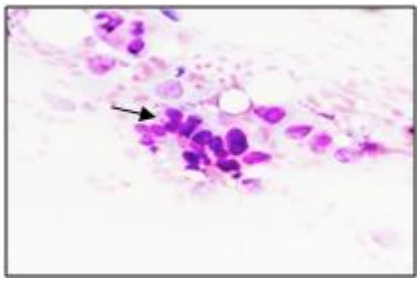

Figure 1: Micronuclei in high power Papanicolaou stain in pleural effusion

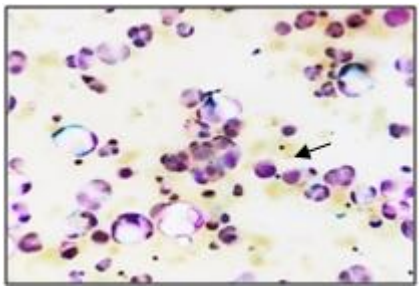

Figure 2: Micronuclei in high power Papanicolaou stain in ascitic effusion

2. Material and Methods

A prospective study conducted on 113 effusion fluids received in the department of pathology, which includes pleural as well as ascitic fluid.

Inclusion Criteria:
All the effusion fluids received in the Department of Pathology during the study period fulfilling the criteria.

Exclusion Criteria: [16]
Clumps of cells with obscured nuclear or cytoplasmic boundaries. Overlapping cells. Degenerated cells.
Apoptotic cells. Cells covered with debris, mucous, bacteria, WBC and RBC. Superimposed lymphocytes and staining artifacts.

Method of Collection:
Fluid samples were received along with relevant clinical history along with quantity, colour, appearance, investigation required and clinical history.

3. Procedure

The effusion fluids were processed in the laboratory, which Involves centrifugation at 1000 rpm for 5 minutes to obtain supernatant and cell pellet. The cell pallet than used to made smear followed by staining with conventional papanicolaou stain. All the prepared smears were examined under light microscopy and micronuclei score per 1000 cells were counted.

The micronuclei were identified using Tolbert et al.(15) Criteria:

- Diameter of MN around 1/3 to 1/16 of diameter of the primary nucleus.
- Shape, colour and texture similar to those of main nucleus.
- Should be in same plane of focus as main nucleus.

Round to oval in shape with no actual contact with nucleus.

- Intensity of staining similar to that of nucleus.
- Cells lying singly were preferred.

4. Results

In our study total no of cases were 113. Among these 14 cases were malignant & 99 were malignant.

Mean age of patients enrolled in present study was 46.7±15.67 years (Range- 13 to 84 years). Majority of patients belonged to age range of 41 to 50 years (27.4%).

The present study documented slight female predominance with male: female ratio of 0.77:1.

Table 1: Distribution of cases according to type of effusion

Characteristic of fluid		Frequency (n=113)	Percentage
Type of fluid	Ascitic	56	49.6
	Pleural	57	50.4
Fluid microscopy formals	Negative	99	87.6
	Positive	14	12.3

Table 2: Distribution of cases according to micronuclei presence

Micronuclei (per 1000 cells)	Frequency (n=113)	Percentage
Absent	82	72.6
Present	31	27.4
Mean±SD	1.04±2.2	
Median (IQR)	0 (0-1.5)	
Range	0-14	

Table 3: Association of mean Micronuclei score with type of fluid and effusions with range

Type of fluid	Benign/ Malignant	Micronuclei Score (MN)		P value
		Mean	Range	
Ascitic	Benign	2.72	1-5	0.001
	Malignant	5.58	2-14	
Pleural	Benign	1.8	1-2	0.001
	Malignant	4.0	2-5	

Table showed MN score among malignant cases ranged from 2 to 14 with mean score 5.58 in Ascitic fluid while 2 to 5 range with mean score of 4.0 in pleural fluid. MN score found higher in malignant as compare to benign effusions.

Micronuclei were present in 31 effusions and among them; - most common diagnosis was gynecological malignancies (35.4%), followed by chronic liver disease with ascites (12.9%). Ca rectum and liver metastasis were reported in 3.2% cases each.

5. Discussion

The integrity of genomic information is one of the fundamental pre-requisite for well being. In cancer cells genomic integrity is disrupted. [16]. One of the manifestations of such genomic instability is formation of micronuclei (MN).[20]. There are currently only few studies of MN scoring on effusion fluids have been done. These studies noticed significant difference in MN scoring between benign and malignant groups of effusions. [17].

Consistently with the literatures, we found that all the cytological smears with a malignant outcome had a significantly higher MN count compared to benign ones. The present study was undertaken to do scoring of MN in effusions and to differentiate effusions as benign and malignant on the basis of MN score.

Mean age of patients enrolled in present study was 46.7±15.67 years (Range- 13 to 84 years). Median age of patients was **46** years (IQR-35-60 years). Majority of patients belonged to age range of 41 to 50 years (27.4%). We found significant correlation between higher age group and micronuclei count. The present study documented slight male predominance with male: female ratio of. We found micronuclei in 31 cases out of 113. In 31 cases there were 23 cases of **ascites** where we found 12 benign and 11 malignant cases. In our study the mean MN score in benign cases was 2.72 with range 1-5, while in malignant cases 5.58 with range of 2-14. In 31 cases there were 8 cases of pleural effusions where 5 were benign and only 3 were malignant. The mean MN score in benign cases was 1.8 with range 1-2, while in malignant cases 4.0 with range of 2-5.

Mean micronuclei score were found to be significantly higher in malignant effusions as compared to benign effusions in both pleural as well as ascetic fluid ($p<0.05$).

Our study shows concordant to the study conducted by **Dravya J. et al. [21]** and Sarojini et al. [16] Dravya J. et all conducted their study on 30 benign as well as 30 malignant cases, where 35 were ascitic effusions and 25 were pleural effusions. They showed mean MN score of 3.77 in malignant effusions, ranging from 0-9.

Another study done by **Sarojini et al [16]** showed mean MN score of 5.86 ranging from 0-38.

Our study has shown the sensitivity of 100% in detecting micronuclei in effusions with specificity of 82.8%. The positive predictive value is 45.2 while negative predictive value 100% in detecting micronuclei. Our study revealed diagnostic accuracy is 84.96%.

Micronuclei were present in 31 effusions and among them, most of the cases were from gynecological malignancies (35.4%), followed by chronic liver disease with ascites. Carcinoma lung and Gastric neoplasm 6.4% each.

Kokenek et al[19] included maximum cases of carcinoma lung followed by malignant mesothelioma.

Dravya J. et al. [21] included carcinoma lung and colorectal carcinoma.

Kaur et al. [18] found most of the cases of metastatic adenocarciomas.

Tyagi et al. [20] included most of the cases of ovarian adenocarcinoma.

6. Limitations of our study

This study was an observational study and did not follow up patients. Evaluation of micronuclei is a time consuming procedure and strict criterion need to be observed to count them in 1000 well preserved cells. Correct identification of micronuclei in smears may be challenging due to excessive chromatin crushing and nuclear debris and mimickers which includes apoptotic bodies, stain deposits, overlapping cells etc.

7. Conclusion

The frequency of micronuclei was more in females when compared to male. There was a linear increase in micronuclei score with increasing age. Increased micronuclei score were observed in malignant effusions than benign effusions. Increased micronuclei frequency is suggestive of individual at high risk of malignant transformation. Micronuclei scoring can be used to distinguish between malignant and benign effusions. The result of our study reveal that there is an absolute, consistent, and proportional relationship between micronuclei score and malignancy in ascitic as well as pleural effusions. This approach can be helpful in the early detection of malignancies in lowresource setting.

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